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A multi-ancestry genome-wide study incorporating gene-smoking interactions identifies multiple new loci for pulse pressure and mean arterial pressure

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Abstract

Elevated blood pressure (BP), a leading cause of global morbidity and mortality, is influenced by both genetic and lifestyle factors. Cigarette smoking is one such lifestyle factor. Across five ancestries, we performed a genome-wide gene-smoking interaction study of mean arterial pressure (MAP) and pulse pressure (PP) in 129,913 individuals in stage 1 and follow-up analysis in 480,178 additional individuals in stage 2. We report here 139 loci significantly associated with MAP and/or PP. Of these, 61 were previously published through main-effect analysis of BP traits, 37 were recently reported by us for SBP and/or DBP through gene-smoking interaction analysis, and 41 were newly identified ($P < 5 \times 10^{-8}$, FDR < 0.05). We also identified 11 new signals near known loci. Eight of the 139 loci showed significant interaction with smoking status. They include *CSMD1* previously reported for insulin resistance and BP in the spontaneously hypertensive rats. Many of the 41 new loci show biologic plausibility for a role in BP regulation. *SLC26A7* encodes a chloride/bicarbonate-exchanger expressed in the renal outer medullary collecting duct. *AVPR1A* is widely expressed including in vascular smooth muscle cells, kidney, myocardium, and brain. *FHADI* is a long non-coding RNA overexpressed in heart failure. *TMEM51* was associated with contractile function in cardiomyocytes. *CASP9* plays a central role in cardiomyocyte apoptosis. Identification of several novel loci was only possible from African ancestry. Our findings highlight the value of multi-ancestry investigations, particularly in studies of interaction with lifestyle factors, where genomic and lifestyle differences may contribute to novel findings.

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Introduction

Elevated blood pressure (BP), a leading cause of morbidity and mortality worldwide, is known to be influenced by both genetic and lifestyle factors. To date genome-wide association studies (GWAS) have identified 578 BP-associated genetic variants residing within about 430 loci.¹⁻⁸ The effects of genetic variants on BP may manifest differently depending on lifestyle exposures. Therefore, incorporating gene-environment (GxE) interactions may identify additional loci.^{9; 10} We established the Gene-Lifestyle Interactions Working Group within the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium in order to assess the impact of interactions with multiple lifestyle factors on the genetics of cardiovascular traits.¹¹ Among many lifestyle factors, cigarette smoking influences BP in both acute¹² and chronic¹³ fashion, motivating genetic association studies of gene-by-smoking interactions.

Recently we reported findings from a genome-wide association meta-analysis incorporating gene-smoking interactions for systolic BP (SBP) and diastolic BP (DBP).¹⁴ In addition to SBP and DBP, blood pressure can also be characterized as having both steady and pulsatile components, each determined by different physiologic properties of the heart and vasculature, and differently related to cardiovascular outcomes. Mean arterial pressure (MAP) reflects the steady component of BP, which is predominantly determined by cardiac output and systemic vascular resistance and regulated by small artery and arteriole tone.¹⁵ Pulse pressure (PP) represents the pulsatile component of BP and is largely determined by cardiac stroke volume and large artery stiffness.^{15; 16} In this study, we performed a genome-wide association meta-analysis of MAP and PP incorporating gene-smoking interactions (**Figure 1**). Here, we report our findings based on 610,091 individuals across five ancestries.

Methods

Participating Studies

Analyses included men and women between 18-80 years of age from European (EUR), African (AFR), Asian (ASN), Hispanic (HIS), and Brazilian (BRZ) ancestries. Forty-eight (48) cohorts consisting of 129,913 individuals (**Table S1**) participated in stage 1 and performed genome-wide analyses. Studies that included data from multiple ancestries (cohorts) contributed multiple analyses, one for each ancestry/cohort. For example, MESA has four cohorts. Seventy-

six (76) additional cohorts consisting of 480,178 individuals (**Table S2**) participated in stage 2 and performed association analyses of 4,373 variants that were identified in stage 1 as either genome-wide significant ($P < 5 \times 10^{-8}$) or suggestive ($P < 10^{-6}$). All participating studies are described in the **Supplemental Notes**. Since discoveries of BP loci to date were largely from EUR populations, considerable effort was made for recruiting most of the available non-EUR cohorts into stage 1 (which limited the availability of non-EUR cohorts in stage 2). Each study obtained informed consent from participants and approval from the appropriate institutional review boards.

Phenotypes and Lifestyle Variables

Resting systolic BP (SBP) and diastolic BP (DBP) were measured using a consistent protocol. For individuals on any anti-hypertensive (BP lowering) medications, 15 mmHg and 10 mmHg were added to their SBP and DBP values, respectively.¹ PP was computed as SBP minus DBP ($PP = SBP - DBP$) and MAP was computed as the sum of DBP and one-third of PP ($MAP = DBP + PP/3$). To reduce the influence of possible outliers, each BP value was winsorized at 6 standard deviations away from the mean (i.e., values greater than 6 SD away from the mean were set at 6 SD).

We considered two of the most widely available smoking variables: ‘current smoking’ status (CurSmk) and ‘ever smoking’ status (EverSmk) (**Table 1**). Current smoking status was defined as 1 if the individual smoked regularly in past year (and as 0 for non-current smokers, which includes both never and former smokers). Ever smoking status was defined as 1 if the individual smoked at least 100 cigarettes during his/her lifetime (and as 0 for the never-smokers). Smoking status was assessed at the time of the BP measurements. Covariates include age, sex, field center (for multi-center studies), and principal component (PC)s (to account for population stratification and admixture). No additional covariates were included. Individuals with missing data for BP, the smoking variable, or any covariates were excluded from analysis. Study-specific summary statistics on phenotypes are presented in **Tables S3-S4**.

Genotype Data

Genotyping was obtained using Illumina (San Diego, CA, USA) or Affymetrix (Santa Clara, CA, USA) genotyping arrays. Each study performed genotype imputation at single nucleotide polymorphisms (SNPs), short insertions and deletions (indels), and larger deletions

that were not genotyped directly but are available from the 1000 Genomes Project.¹⁷ For imputation, most studies used the 1000 Genomes Project Phase I Integrated Release Version 3 Haplotypes (2010-11 data freeze, 2012-03-14 haplotypes), which contain haplotypes of 1,092 individuals of all ancestry backgrounds. Study-specific information on genotyping and imputation is presented in **Tables S5-S6**.

Cohort-specific Analysis

Participating studies performed association analyses separately within each ancestry for MAP and PP incorporating current smoking (CurSmk) and ever smoking (EverSmk). All studies performed regression analysis using a model with both genetic main and GxE interaction effects¹⁸ and provided the estimated genetic main and interaction effects and a robust estimate of the corresponding covariance matrix. In addition, studies in stage 1 performed regression analyses with the genetic main-effect model, in the exposed (Smk=1) and unexposed strata (Smk=0) separately, and provided estimates of the stratum-specific effects and robust estimates of their standard errors (SE). Either sandwich¹⁹ or ProbABEL²⁰ packages were used to obtain robust estimates of covariance matrices and robust SEs for samples of unrelated individuals. Family studies used the generalized estimating equations (GEE) approach, treating each family as a cluster, or the linear mixed effect model approach with a random polygenic component (for which the covariance matrix depends on the kinship matrix). Robust estimates of covariance matrices and SEs were used to safeguard against mis-specification of the mean model and violation of the assumption of constant BP variance across smoking groups (heteroscedasticity).^{21; 22}

Quality Control (QC)

We performed extensive QC using the R package EasyQC²³ for all cohort-specific results. For GWAS results in stage 1, each cohort applied a preliminary filter on their imputed data excluding variants with minor allele frequency (MAF) < 1%. Variants with imputation quality measure < 0.5 were subsequently excluded. We performed the “study-level” QC, which included carefully checking the observed allele frequencies against the corresponding ancestry-specific 1000 Genomes Project data and harmonizing marker names to ensure consistencies across cohorts. In addition, in stage 1, we compared results from the joint and stratified models, as explained elsewhere.²⁴ To identify cross-study issues, we then performed the “meta-level” QC by checking result files across all cohorts for each analysis. This included visually comparing

summary statistics (mean, median, inter-quartile range, etc.) on all effect estimates, SEs and p-values, and examining SE-N and QQ plots to reveal issues with trait transformation²³ or other analytical problems. Encountered QC problems were communicated and resolved with the individual cohorts. More detailed information about QC is described elsewhere.^{11; 14}

Meta-analyses

After selecting high-quality variants through extensive QC, approximately 18.8 million SNPs and small insertion and deletion (indels) variants were included in the meta-analysis (the number of variants varied across the ancestry groups). To combine cohort-specific results within each ancestry, we first performed ancestry-specific meta-analyses; the results were then combined through meta-analysis to obtain evidence of “trans-ancestry” association. Inverse-variance weighted meta-analysis with METAL²⁵ was used for the 1 degree of freedom (DF) test of interaction effect (with $H_0: \beta_{GE} = 0$). For 2 DF tests of both SNP main and interaction effects (with $H_0: \beta_G = \beta_{GE} = 0$), the joint meta-analysis of Manning et al²⁶ was used. In the stratified model, we performed meta-analysis using the approach of Randall et al²⁷ for the 1 DF test and the approach of Aschard et al²⁸ for the 2 DF test using the R package EasyStrata.²⁹ Additional details about the meta-analytic approach are described elsewhere.²⁴

In stage 1, genomic control correction³⁰ was applied twice, first for cohort-specific GWAS results if their genomic control lambda value was greater than 1, and again after the meta-analysis. Variants that passed QC were excluded if they were represented in fewer than 5,000 samples from at least 3 cohorts. Variants that were genome-wide significant ($P < 5 \times 10^{-8}$) or suggestive ($P < 1 \times 10^{-6}$) in stage 1 were pursued in stage 2. Heterogeneity p-values at the selected variants were $> 1 \times 10^{-5}$, indicating limited heterogeneity (data not shown). In stage 2, genomic control correction was not applied to the replication statistics as association analysis was performed only at select variants. Meta-analysis combining results of stages 1 and 2 was also performed. In addition, genome-wide significant variants in stage 1 were tested for formal replication in stage 2 using Bonferroni-corrected significance threshold.

Genome-wide Significant Variants

We considered a variant with $P < 5 \times 10^{-8}$ to be genome-wide significant. False discovery rate (FDR) q-values were computed using the R function p.adjust using the step-up method by Benjamini and Hochberg.³¹ To identify a set of independent (index) variants, we performed the

linkage disequilibrium (LD)-based clumping procedure using PLINK³² and EasyStrata.²⁹ A locus is defined through LD-based clumping that uses physical distance (± 1 Mb) and LD threshold of $r^2 > 0.1$. Since valid methods do not exist for conditional analysis involving interactions across multi-ancestry studies, we relied on a relatively more stringent LD threshold ($r^2 > 0.1$) for identifying “independent” loci. For LD reference, ancestry-specific 1000 Genomes Project data were used for ancestry-specific results and the entire cosmopolitan dataset was used for trans-ancestry results.

BP Variance Explained

We computed BP variance explained by genome-wide results, based on stage 1 stratified results with current-smoking status in European ancestry.³³ Within each of the smoking strata, we computed the variance of MAP and PP explained by subsets of variants selected using 15 significance thresholds ranging from 1×10^{-8} to 0.1

Functional Inferences

We conducted DEPICT analyses³⁴ based on genome-wide significant ($P < 5 \times 10^{-8}$) variants from the combined analysis of stages 1 and 2. DEPICT performs three consecutive analyses: i) gene prioritization at the identified loci; ii) gene set enrichment analyses; and iii) tissue- and cell-type specific expression analyses. To obtain input for the analyses, DEPICT applied a combined distance and LD based threshold (500 kb flanking regions and LD $r^2 > 0.1$) between the identified variants and the 1000 Genomes reference data³⁵. A further clumping (LD $r^2 > 0.5$ between the non-overlapping variants and known functional coding or cis-acting regulatory variants) was used to obtain a list of genes overlapping with the identified variants. The major histocompatibility complex region on chromosome 6 (25 Mb – 35 Mb) was removed for further analyses.

For gene prioritization, DEPICT compared functional similarity of genes across identified loci using a gene score, which was adjusted for confounders like gene length. To obtain FDR, the scoring was repeated 50 times based on 500 pre-compiled null GWAS. For gene-set enrichment analyses, DEPICT used 14,461 pre-compiled reconstituted gene sets; they include 737 Reactome pathways, 2,473 phenotypic gene sets (derived from the Mouse Genetics Initiative), 184 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, 5,083 Gene Ontology terms, and 5,984 protein molecular pathways (derived from protein-protein

interactions). For tissue and cell type enrichment analyses, DEPICT used expression data from the 209 MeSH annotations for 37,427 microarrays of the Affymetrix U133 Plus 2.0 Array platform.

Results

Overview

Through genome-wide search of meta-analyses with 129,913 individuals in stage 1, we identified 1,692 significant ($P \leq 5 \times 10^{-8}$) and 2,681 suggestive ($P \leq 10^{-6}$) variants associated with MAP and/or PP. We performed meta-analysis combining stages 1 and 2 (Manhattan plots **Figure S1**; Quantile-quantile, QQ, plots **Figure S2**). Through this combined analysis with 610,091 individuals, we identified 41 novel genome-wide significant loci ($P \leq 5 \times 10^{-8}$) that are at least 1Mb away from any of known BP loci (**Table 2**). We also identified 11 additional new signals near (but not in LD, $r^2 < 0.1$, with) known BP loci (**Table 3**). LocusZoom plots are shown in **Figure S3**. FDR q-values provided additional evidence for these newly-identified loci (FDR < 0.01 for 48 of the 52; and FDR < 0.05 for all 52 loci).

Among the 81 loci we reported recently through gene-smoking interactions for SBP and/or DBP¹⁴, thirty-seven (37) were also associated with MAP and/or PP (**Figure 2, Table S7**). Among the previously published loci through main effects GWAS for any BP trait,¹⁻⁸ sixty-one (61) loci showed association with MAP and PP as well. Among the genome-wide significant variants in stage 1, which resided in 112 independent loci, 53 loci were formally replicated in stage 2 using Bonferroni-adjusted significance levels ($P < 0.05/112$). All of them had been identified previously; 44 through main effects GWAS,¹⁻⁸ and 9 through gene-smoking interaction analysis we reported recently for SBP and DBP.¹⁴

The Role of Interactions

In total, we identified 139 loci and 11 new signals significantly-associated with MAP and/or PP: 41 new loci, 11 new signals near known BP loci, 37 recently reported by us for SBP and/or DBP,¹⁴ and 61 previously published through main-effect GWAS analysis of BP traits.¹⁻⁸ Among them, variants at eight loci showed genome-wide significant interactions (1 DF interaction $P < 5 \times 10^{-8}$) with smoking status (**Figure 3**). All 8 loci were identified with current smoking status; these variants have larger effects in current smokers than in non-current

smokers. Five interactions were newly identified (**Table 2**), and the other 3 were previously reported for SBP or DBP (**Table S7**). These variants showing interaction effects were identified only in individuals of African ancestry in stage 1. Because of the limited sample size of African ancestry in stage 2, no replication of their interactions was attempted in stage 2.

BP Variance Explained

Within each of the smoking strata, we computed the variance of MAP and PP explained by genome-wide results³³ in European ancestry (**Figure 4**). The independent set of variants, 38 for MAP and 12 for PP, with $P \leq 5 \times 10^{-8}$ explained 1.9% of variance in MAP and 0.5% of variance in PP. The difference in explained variance between the smokers and non-smokers was not significant, suggesting that BP variance explained by interaction effects is very small. Similar inference was observed with the results from ever-smoking status (data not shown).

Functional Inferences

To obtain functional annotations from HaploReg,³⁶ we focused on the index variants representing the 89 loci (41 novel loci, 11 new signals near known loci, and 37 recently reported) that showed association with MAP and/or PP. There was one missense variant, rs1009382. Of the remaining non-coding variants (37 intronic and 51 intergenic), 15 were in promoter histone marks, 47 in enhancer histone marks, 28 in DNase I marks, and 8 altered the binding sites of regulatory proteins (**Table S8**). Using GERP³⁷, 5 variants were identified as being conserved among vertebrates, with 3 variants identified as such using SiPhy.³⁸ For 27 variants, *cis*-eQTL evidence was available with varying degrees of association with expression probes. In particular, 10 of them were identified by GTEx³⁹ as *cis*-eQTLs across various tissues (**Table S9**). In addition, we obtained information on microarray-based gene and exon expression levels in whole blood from over 5,000 individuals of the Framingham Heart Study⁴⁰ (**Table S10**). There were 109 variant-transcript pairs (representing 26 variants) with *cis*-eQTL evidence (at $P < 8.9 \times 10^{-5}$, FDR < 0.002). Among 26 variants, the 3 variants had the most significant eQTL evidence: rs112947839, rs1009382, and rs7753826 associated with 21, 18, and 10 transcripts, respectively.

The DEPICT analyses prioritized genes (FDR < 5%) at 40 loci, including 16 genes that did not match the nearest gene of the identified lead variant (**Table S11**). Furthermore, the analyses highlighted 56 significantly (FDR < 5%) enriched gene sets. Many of these highlight cardiovascular mechanisms, such as ‘abnormal blood vessel morphology’, ‘thin myocardium’ or

‘abnormal heart development’, **Table S12**). We also observed that genome-wide significant MAP and PP loci are enriched for genes expressed in the ileum (**Table S13**).

Associations of BP Loci with Cardiometabolic Traits

We obtained association results of the 89 index variants associated with MAP or PP (representing 41 novel loci, 11 new signals near known loci, and 37 recently reported loci) with multiple cardiometabolic traits: coronary artery disease (CAD), stroke, adiposity, diabetes, and renal function (**Tables S14-S19**). For 36 out of 47 scenarios (highlighted in red, **Table S20**), the observed number of variants with nominal evidence of association ($P < 0.05$) was higher than that expected by chance alone ($P_{\text{Binomial}} < 0.05/11$, corrected for 11 traits used in the lookups). For example, we observed 7 and 11 such associations with CAD and myocardial infarction, respectively, where the expected count is 2.2 for both traits. Corroborating evidence of the multiple cardiometabolic traits were found for the eight of the 52 new loci: rs2071405, *AGT*; rs146622638, *GPM6A*; rs1009382, *TNXB*; rs6601302, *LOC157273*; rs7005363, *MSRA*; rs12156238, *FAM167A*; rs1010064, *LOC100506393*; rs201028933, *LOC338758*. These overlapping signals support that these traits may share a common pathophysiology.

Loci Overlapping with Previously Reported SBP or DBP Loci

Among the loci that were reported by us recently as significantly associated with SBP and/or DBP based on gene-by-smoking interaction analysis,¹⁴ thirty-seven (37) were also associated with MAP and/or PP (**Table S7**). Among them, 9 loci were formally replicated in stage 2 and showed association with all 4 BP traits. Variants at these nine loci were all also genome-wide significant in the combined analysis of stages 1 and 2 in individuals of European ancestry. For variants at six of the nine loci, there was supporting evidence of association in individuals of non-European ancestry, which resulted in stronger statistical significance from trans-ancestry analysis. One such locus was rs351364 (in *WNT2B*), where only trans-ancestry analysis reached genome-wide significance in stage 1; the direction of the genetic effect was consistent across all ancestries.

New Signals near Known BP Loci

Eleven (11) new signals were identified near known BP loci (but not in LD, $r^2 < 0.1$). One such signal was rs140881076 (chr1:15364113, **Figure 5A**) in association with PP in individuals of African ancestry. This signal is 434 kb away and in complete linkage equilibrium

with *CELA2A* locus (rs3820068, chr1:15798197) which was recently identified in individuals of European ancestry.^{7; 8} Several nearby genes have been implicated in cardiovascular traits. *FHADI* is a long non-coding RNA overexpressed in heart failure,⁴¹ *TMEM51* has been associated with contractile function in cardiomyocytes,⁴² and *CASP9* plays a central role in cardiomyocyte apoptosis.⁴³ A candidate gene study identified a missense mutation in *CASP9* as associated with ischemic stroke in Koreans,⁴⁴ Differential methylation patterns in *TMEM51* have also been described in peripheral blood leukocytes of smokers.^{45; 46}

Through trans-ancestry analysis, we identified one locus (rs1010064) associated with both MAP and PP. This is ~500 kb upstream of but not in LD with *PDE3A*, a known BP gene with a role in regulating growth in vascular smooth muscle cells.^{4; 47} Missense mutations in *PDE3A* have been linked with autosomal dominant syndrome characterized by treatment-resistant hypertension and brachydactyly.^{48; 49} SNPs in this locus have also shown suggestive associations with aortic root diameter,⁵⁰ resistant hypertension,⁵¹ and SBP in a SNP-alcohol consumption interaction analysis.⁵²

New BP Loci

Several genes near the 41 novel loci show biologic plausibility for a role in BP regulation. One such gene is *CSMD1* (rs140994551, chr8:4449086, associated with PP in individuals of African ancestry while considering interaction with current smoking status, **Figure 5B**). In animal models, variants in *CSMD1* were associated with both insulin resistance and BP in the spontaneously hypertensive rats.⁵³ In humans, there was suggestive evidence of association with hypertension in two Korean cohorts,⁵⁴ with peripheral artery disease in a Japanese population,⁵⁵ with waist-hip ratio adjusted for BMI in men,⁵⁶ with insulin resistance in African Americans,⁵⁷ and with studies of addiction and related disorders.⁵⁸ Another new locus is *LRRC69* (rs11991823, chr8:92188440, associated with PP, identified through trans-ancestry analysis, **Figure 5C**). A copy number variant in this gene has been shown to be weakly associated ($P = 0.04$) with BP in a Korean population.⁵⁹ The nearby gene *SLC26A7* encodes a chloride/bicarbonate-exchanger expressed specifically in the renal outer medullary collecting duct.⁶⁰ Two PP loci include genes involved in the NF κ B signaling pathway (*TNFRSF11A* and *NFIB*). This inflammatory pathway has been implicated in hypertension-induced renal dysfunction in murine models,⁶¹ and with endothelial dysfunction in overweight/obese and older

humans.⁶² There was suggested evidence of association of variants in *TNFRSF11A* with BP traits in Chinese women.⁶³

A new locus near *AVPR1A* (rs146924684 chr12:63437286, associated with MAP, **Figure 5D**) also has strong biologic plausibility. Vasopressin is an antidiuretic hormone and a potent vasoconstrictor that exerts its effect through activation of a family of receptors, including the arginine vasopressin receptor subtype 1A (*AVPR1A*) which is widely expressed including in vascular smooth muscle cells, kidney, myocardium, and brain.⁶⁴ In glomerular macula densa cells, *AVPR1A* facilitates activation of the renin-angiotensin-aldosterone system and increases expression of the aquaporin 2 water channel.⁶⁵ *AVPR1A* stimulation is also necessary for maintaining normal BP; in murine knockout models, basal BP is significantly decreased and the arterial baroreceptor reflex markedly impaired.⁶⁶ Notably, there is data to support a role for vasopressin not only in the maintenance, but also in the development, of hypertension. Vasopressin receptor 1A blockade in young, still normotensive, spontaneously hypertensive rats (SHR) attenuates the later development of hypertension in adult SHR despite withdrawal of drug therapy.⁶⁷

New Loci Related to the Reported BP Regulation

We identified several loci with potential relevance to the structure and function of primary cilia, in addition to those we reported recently.¹⁴ Three PP-associated loci were near genes implicated with nephronophthisis, including those with mutations linked to Bardet-Biedl Syndrome (*BBS7* and *MYO3A*) and with Joubert Syndrome (*AHII*). Another PP-associated locus was near *NEDD4L*, which encodes the E3 ubiquitin ligase NEDD4-2 and has been shown to regulate a renal epithelial sodium channel (ENaC/*SCNN1*) that is critical for maintenance of sodium homeostasis.⁶⁸ ENaC is the channel responsible for the monogenetic disorder of BP regulation, Liddle Syndrome. Loss of NEDD4-2 in the renal tubules results in increased activity of the ENaC channel, resulting in salt-sensitive hypertension.⁶⁹ Candidate gene studies identified variants in *NEDD4L* as associated with sodium lithium countertransport,⁷⁰ hypertension,⁷¹ treatment response to β -blockers, and diuretics in hypertensive patients.⁷¹⁻⁷³

We identified two additional loci with potential relevance to the dopaminergic system, in addition to those we reported recently.¹⁴ Dopamine signaling plays a key role in both central and peripheral BP regulation.⁷⁴⁻⁷⁶ A regulatory subunit (*PPP2R2A*) of the dopamine receptor 2R

(D2R) was associated with MAP. In murine renal proximal tubule cells, inhibition of this regulatory protein leads to increased expression of markers of renal inflammation and injury.⁷⁷ A newly identified MAP-associated locus *SESN2* is also related to the dopaminergic system; activation of the D2R has been shown to increase the expression of *SESN2*, which protects the kidney against renal oxidative stress.⁷⁸ *SESN2* also protects endothelial cell lines against angiotensin II-induced endothelial toxicity.⁷⁹ Two additional loci include genes involved in dopamine signaling (*ATP13A2*⁸⁰ and *ARPP21*⁸¹). Activation of dopamine centers of the brain has also been implicated in drug and nicotine abuse.⁸²

In addition, we found a PP-associated locus near *SDHB*, which encodes the mitochondrial protein succinate dehydrogenase. Variants in this gene have been identified in individuals with carotid body tumors and pheochromocytomas/paragangliomas, endocrine tumors that secrete dopamine and/or norepinephrine and can modulate BP regulation even when tumors are not clinically apparent.^{83; 84} Variants near this locus have been marginally associated with DBP in pre-pubertal European children.⁸⁵ Tyrosinase (with its related protein, TYRP1) catalyzes the first rate-limiting step in pathway in the formation of L-Dopa.⁸⁶ Although variants in *TYRP1* were suggestively associated with SBP by the International Consortium for Blood Pressure,⁸⁷ we identified this locus as associated with PP at genome-wide significance.

Discussion

Mean arterial pressure (MAP) measures the steady component, which is a function of the left ventricular contractility, heart rate, small-artery resistance and vascular elasticity averaged over time.¹⁵ Pulse pressure (PP) measures the pulsatile component, which is a function of the left ventricular stroke volume, large-artery stiffness, early pulse wave reflection, and heart rate.⁸⁸ These BP traits not only differ in their physiologic properties, but are also differently related to cardiovascular outcomes.^{15; 88-90} Our genome-wide association meta-analysis incorporating gene-smoking interactions identified 139 loci significantly-associated with MAP and/or PP: 61 were previously published through main-effect GWAS analysis,¹⁻⁸ 37 were recently reported by us for SBP and/or DBP through gene-smoking interaction analysis,¹⁴ 41 are newly reported here. Our analysis also identified 11 new signals near known BP loci (but not in LD, $r^2 < 0.1$).

Among the loci significantly associated with MAP and/or PP, 8 loci showed significant interaction with smoking status from the 1 DF interaction tests. At these 8 loci, the joint 2 DF P-values ranged from 1×10^{-7} to 5×10^{-11} , indicating that loci were identified mostly because of their interaction with smoking status. One such locus is *CSMD1*. While variants of this gene were previously suggested for addiction and related disorders,⁵⁸ we identified this locus at genome-wide significance (1 DF P = 4.3×10^{-9} , 2 DF P = 2.1×10^{-11}). In our study, another locus near *AHR* showed weak evidence of interaction with smoking (1 DF P = 1.6×10^{-4} , 2 DF P = 1.7×10^{-9} associated with MAP). Variants in *AHR* are shown to interact with variants in *CYP1A1*, a detoxifying enzyme, to explain BP differences between smokers and non-smokers.⁹¹ *AHR* encodes a ligand-activated transcription factor, and *AHR* knock-out mice have increased MAP and ventricular hypertrophy/fibrosis with increased plasma levels of angiotensin II.⁹² Given the evidence that environmental toxins, including tobacco smoke, activate AHR, it is pertinent to note that AHR, in turn, activates tyrosinase activity, the rate limiting step for L-dopa biosynthesis.⁸⁶ Activation of the AHR protein represses T-cadherin expression, which functions as a negative growth regulator in vascular smooth muscle cells.^{93; 94} T-cadherin (encoded by *CDH13*) has been previously identified as a BP susceptibility locus.⁹⁵ Notably, while the endogenous ligand for AHR remains uncertain,⁹⁶ exogenous ligands include polycyclic aromatic hydrocarbons which are found in tobacco smoke and other environmental pollutants.⁹⁷

We found that most of MAP-associated loci were previously associated with SBP and/or DBP. This is not surprising given that MAP is closely related physiologically to SBP and DBP. In contrast, analysis of PP yielded a greater number of novel significant loci that are unique to PP. Loci associated with PP may be identifying different physiologic processes than loci associated with MAP, SBP and DBP. For example, the steady component of BP can be effectively targeted by β -adrenergic receptor and calcium-channel blockers that both modulate arteriolar tone. Angiotensin converting enzyme (ACE) inhibitors, which favor remodeling of vascular connective tissue, may impact PP to a greater extent.⁹⁸ This is a clinically important concept since hypertension is often more effectively treated by combination drug therapy to target different physiologic pathways.⁹⁹

This large-scale multi-ancestry study has some limitations. First, because most of the known BP loci were identified in European and Asian ancestries, considerable effort was made

to recruit most of the available studies from the other ancestries into stage 1. Although we were able to identify several new loci in African ancestry, the relatively smaller stage 2 sample size of African ancestry ($n=7,786$) has limited our ability to replicate these new loci. Second, some of our new loci identified through the 2DF joint test may have been identified due to a main effect because of a larger sample size and more diverse ancestries, not necessarily from gene-smoking interaction. Unfortunately, we are unable to verify this because analysis of main effects alone, without regard to smoking status, was not performed. Third, conditional analysis (such as GCTA) based on summary statistics was not performed because valid methods do not currently exist for GxE interactions. In order to infer independence of the loci, we pursued clumping as defined by PLINK³² and used stringent thresholds (± 1 Mb and weak LD with $r^2 < 0.1$). Fourth, if there is a gene-environment correlation, a potential confounding of GxE with interaction between covariate and smoking exposure may exist. This can inflate Type I error of the GxE interaction test.¹⁰⁰

In summary, this study identified 41 new loci and 11 new signals near known BP loci that are uniquely associated with MAP and/or PP, demonstrating the promise of gene-lifestyle interactions for genetic and environmental dissection of BP traits. Variants in several loci were identified in individuals of African ancestry, highlighting the importance of genetic studies in diverse populations. These African-specific loci were not replicated in stage 2 because of limited sample size and, therefore, require further validation. Many of these new loci (including *CSMD1*, *TMEM51*, *SLC26A7*, *TNFRSF11A*, and *AVPR1A*) show biologic plausibility for a role in BP regulation. They include additional loci of potential relevance to the structure and function of primary cilia and the dopaminergic system. Eight out of 133 significant loci showed significant interaction with smoking status. Because some interactions may be driven by other lifestyle factors that are correlated with smoking, a follow-up study such as Tyrrell and her colleague¹⁰¹ that jointly examines multiple lifestyle factors can shed light on further understanding of the nature of the smoking interaction effects on BP. Our findings highlight the value of multi-ancestry investigations, particularly in studies of interaction with lifestyle factors, where genomic and lifestyle differences may contribute to novel findings.

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Web Resources

DEPICT, <https://data.broadinstitute.org/mpg/depict/>; GTEx, gtexportal.org; LocusZoom, <http://locuszoom.sph.umich.edu/>; HaploReg, http://www.broadinstitute.org/mammals/haploreg/haploreg_v3.php; METAL, http://genome.sph.umich.edu/wiki/METAL_Documentation; RegulomeDB, <http://regulomedb.org/>; Roadmap Epigenomics, <http://www.roadmapepigenomics.org/>.

References

1. Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M.D., Bochud, M., Coin, L., Najjar, S.S., Zhao, J.H., Heath, S.C., Eyheramendy, S., et al. (2009). Genome-wide association study identifies eight loci associated with blood pressure. *Nature genetics* 41, 666-676.
2. Levy, D., Ehret, G.B., Rice, K., Verwoert, G.C., Launer, L.J., Dehghan, A., Glazer, N.L., Morrison, A.C., Johnson, A.D., Aspelund, T., et al. (2009). Genome-wide association study of blood pressure and hypertension. *Nature genetics* 41, 677-687.
3. Ehret, G.B., Munroe, P.B., Rice, K.M., Bochud, M., Johnson, A.D., Chasman, D.I., Smith, A.V., Tobin, M.D., Verwoert, G.C., Hwang, S.J., et al. (2011). Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 478, 103-109.
4. Ehret, G.B., Ferreira, T., Chasman, D.I., Jackson, A.U., Schmidt, E.M., Johnson, T., Thorleifsson, G., Luan, J., Donnelly, L.A., Kanoni, S., et al. (2016). The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nature genetics* 48, 1171-1184.
5. Liu, C., Kraja, A.T., Smith, J.A., Brody, J.A., Franceschini, N., Bis, J.C., Rice, K., Morrison, A.C., Lu, Y., Weiss, S., et al. (2016). Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. *Nature genetics* 48, 1162-1170.
6. Surendran, P., Drenos, F., Young, R., Warren, H., Cook, J.P., Manning, A.K., Grarup, N., Sim, X., Barnes, D.R., Witkowska, K., et al. (2016). Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. *Nature genetics* 48, 1151-1161.
7. Hoffmann, T.J., Ehret, G.B., Nandakumar, P., Ranatunga, D., Schaefer, C., Kwok, P.Y., Iribarren, C., Chakravarti, A., and Risch, N. (2016). Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation. *Nature genetics*.
8. Warren, H. (2017). Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. *Nature genetics*.
9. Manning, A.K., Hivert, M.F., Scott, R.A., Grimsby, J.L., Bouatia-Naji, N., Chen, H., Rybin, D., Liu, C.T., Bielak, L.F., Prokopenko, I., et al. (2012). A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. *Nature genetics* 44, 659-669.
10. Kirk, E.P. (2017). Genes, Environment, and the Heart: Putting the Pieces Together. *Circulation Cardiovascular genetics* 10.
11. Rao, D.C., Sung, Y.J., Winkler, T.W., Schwander, K., Borecki, I., Cupples, L.A., Gauderman, W.J., Rice, K., Munroe, P.B., and Psaty, B. (2017). A Multi-ancestry study of gene-lifestyle interactions for cardiovascular traits in 610,475 individuals from 124 cohorts: Design and rationale. *Circulation Cardiovascular genetics* 10, e001649.
12. Mann, S.J., James, G.D., Wang, R.S., and Pickering, T.G. (1991). Elevation of ambulatory systolic blood pressure in hypertensive smokers. A case-control study. *JAMA : the journal of the American Medical Association* 265, 2226-2228.
13. Primatesta, P., Falaschetti, E., Gupta, S., Marmot, M.G., and Poulter, N.R. (2001). Association between smoking and blood pressure: evidence from the health survey for England. *Hypertension* 37, 187-193.
14. Sung, Y.J., Winkler, T.W., de Las Fuentes, L., Bentley, A.R., Brown, M.R., Kraja, A.T., Schwander, K., Ntalla, I., Guo, X., Franceschini, N., et al. (2018). A Large-Scale Multi-ancestry Genome-wide Study Accounting for Smoking Behavior Identifies Multiple Significant Loci for Blood Pressure. *American journal of human genetics* 102, 375-400.

15. Franklin, S.S., Gustin, W.t., Wong, N.D., Larson, M.G., Weber, M.A., Kannel, W.B., and Levy, D. (1997). Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation* 96, 308-315.
16. Dart, A.M., and Kingwell, B.A. (2001). Pulse pressure--a review of mechanisms and clinical relevance. *J Am Coll Cardiol* 37, 975-984.
17. 1000 Genomes Project Consortium, Abecasis, G.R., Auton, A., Brooks, L.D., DePristo, M.A., Durbin, R.M., Handsaker, R.E., Kang, H.M., Marth, G.T., and McVean, G.A. (2012). An integrated map of genetic variation from 1,092 human genomes. *Nature* 491, 56-65.
18. Kraft, P., Yen, Y.C., Stram, D.O., Morrison, J., and Gauderman, W.J. (2007). Exploiting gene-environment interaction to detect genetic associations. *Human heredity* 63, 111-119.
19. Zeileis, A. (2006). Object-oriented computation of sandwich estimators. *J Stat Softw* 16.
20. Aulchenko, Y.S., Struchalin, M.V., and van Duijn, C.M. (2010). ProbABEL package for genome-wide association analysis of imputed data. *BMC bioinformatics* 11, 134.
21. Tchetgen Tchetgen, E.J., and Kraft, P. (2011). On the robustness of tests of genetic associations incorporating gene-environment interaction when the environmental exposure is misspecified. *Epidemiology* 22, 257-261.
22. Voorman, A., Lumley, T., McKnight, B., and Rice, K. (2011). Behavior of QQ-plots and genomic control in studies of gene-environment interaction. *PloS one* 6, e19416.
23. Winkler, T.W., Day, F.R., Croteau-Chonka, D.C., Wood, A.R., Locke, A.E., Magi, R., Ferreira, T., Fall, T., Graff, M., Justice, A.E., et al. (2014). Quality control and conduct of genome-wide association meta-analyses. *Nature protocols* 9, 1192-1212.
24. Sung, Y.J., Winkler, T.W., Manning, A.K., Aschard, H., Gudnason, V., Harris, T.B., Smith, A.V., Boerwinkle, E., Brown, M.R., Morrison, A.C., et al. (2016). An Empirical Comparison of Joint and Stratified Frameworks for Studying G x E Interactions: Systolic Blood Pressure and Smoking in the CHARGE Gene-Lifestyle Interactions Working Group. *Genetic epidemiology* 40, 404-415.
25. Willer, C.J., Li, Y., and Abecasis, G.R. (2010). METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 26, 2190-2191.
26. Manning, A.K., LaValley, M., Liu, C.T., Rice, K., An, P., Liu, Y., Miljkovic, I., Rasmussen-Torvik, L., Harris, T.B., Province, M.A., et al. (2011). Meta-analysis of gene-environment interaction: joint estimation of SNP and SNP x environment regression coefficients. *Genetic epidemiology* 35, 11-18.
27. Randall, J.C., Winkler, T.W., Kutalik, Z., Berndt, S.I., Jackson, A.U., Monda, K.L., Kilpelainen, T.O., Esko, T., Magi, R., Li, S., et al. (2013). Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS genetics* 9, e1003500.
28. Aschard, H., Hancock, D.B., London, S.J., and Kraft, P. (2010). Genome-wide meta-analysis of joint tests for genetic and gene-environment interaction effects. *Human heredity* 70, 292-300.
29. Winkler, T.W., Kutalik, Z., Gorski, M., Lottaz, C., Kronenberg, F., and Heid, I.M. (2015). EasyStrata: evaluation and visualization of stratified genome-wide association meta-analysis data. *Bioinformatics* 31, 259-261.
30. Devlin, B., and Roeder, K. (1999). Genomic control for association studies. *Biometrics* 55, 997-1004.
31. Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *J Roy Stat Soc B Met* 57, 289-300.
32. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., et al. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *American journal of human genetics* 81, 559-575.

33. Kutalik, Z., Whittaker, J., Waterworth, D., consortium, G., Beckmann, J.S., and Bergmann, S. (2011). Novel method to estimate the phenotypic variation explained by genome-wide association studies reveals large fraction of the missing heritability. *Genetic epidemiology* 35, 341-349.
34. Pers, T.H., Karjalainen, J.M., Chan, Y., Westra, H.-J., Wood, A.R., Yang, J., Lui, J.C., Vedantam, S., Gustafsson, S., Esko, T., et al. (2015). Biological interpretation of genome-wide association studies using predicted gene functions. *Nature communications* 6, 5890-5890.
35. Genomes Project, C., Abecasis, G.R., Altshuler, D., Auton, A., Brooks, L.D., Durbin, R.M., Gibbs, R.A., Hurles, M.E., and McVean, G.A. (2010). A map of human genome variation from population-scale sequencing. *Nature* 467, 1061-1073.
36. Ward, L.D., and Kellis, M. (2012). HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 40, D930-934.
37. Davydov, E.V., Goode, D.L., Sirota, M., Cooper, G.M., Sidow, A., and Batzoglou, S. (2010). Identifying a high fraction of the human genome to be under selective constraint using GERP++. *PLoS Comput Biol* 6, e1001025.
38. Garber, M., Guttman, M., Clamp, M., Zody, M.C., Friedman, N., and Xie, X. (2009). Identifying novel constrained elements by exploiting biased substitution patterns. *Bioinformatics* 25, i54-62.
39. Consortium, G.T. (2015). Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 348, 648-660.
40. Joehanes, R., Zhang, X., Huan, T., Yao, C., Ying, S.X., Nguyen, Q.T., Demirkale, C.Y., Feolo, M.L., Sharopova, N.R., Sturcke, A., et al. (2017). Integrated genome-wide analysis of expression quantitative trait loci aids interpretation of genomic association studies. *Genome biology* 18, 16.
41. di Salvo, T.G., Yang, K.C., Brittain, E., Absi, T., Maltais, S., and Hemnes, A. (2015). Right ventricular myocardial biomarkers in human heart failure. *J Card Fail* 21, 398-411.
42. Cingolani, O.H., Kirk, J.A., Seo, K., Koitabashi, N., Lee, D.I., Ramirez-Correa, G., Bedja, D., Barth, A.S., Moens, A.L., and Kass, D.A. (2011). Thrombospondin-4 is required for stretch-mediated contractility augmentation in cardiac muscle. *Circ Res* 109, 1410-1414.
43. Han, Y., Chen, Y.S., Liu, Z., Bodyak, N., Rigor, D., Bisping, E., Pu, W.T., and Kang, P.M. (2006). Overexpression of HAX-1 protects cardiac myocytes from apoptosis through caspase-9 inhibition. *Circ Res* 99, 415-423.
44. Lee, B.Y., Chon, J., Kim, H.S., Lee, J.H., Yun, D.H., Yoo, S.D., Kim, D.H., Lee, S.A., Han, Y.J., Lee, H., et al. (2017). Association Between a Polymorphism in CASP3 and CASP9 Genes and Ischemic Stroke. *Ann Rehabil Med* 41, 197-203.
45. Elliott, H.R., Tillin, T., McArdle, W.L., Ho, K., Duggirala, A., Frayling, T.M., Davey Smith, G., Hughes, A.D., Chaturvedi, N., and Relton, C.L. (2014). Differences in smoking associated DNA methylation patterns in South Asians and Europeans. *Clin Epigenetics* 6, 4.
46. Markunas, C.A., Xu, Z., Harlid, S., Wade, P.A., Lie, R.T., Taylor, J.A., and Wilcox, A.J. (2014). Identification of DNA methylation changes in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 122, 1147-1153.
47. Begum, N., Hockman, S., and Manganiello, V.C. (2011). Phosphodiesterase 3A (PDE3A) deletion suppresses proliferation of cultured murine vascular smooth muscle cells (VSMCs) via inhibition of mitogen-activated protein kinase (MAPK) signaling and alterations in critical cell cycle regulatory proteins. *J Biol Chem* 286, 26238-26249.
48. Maass, P.G., Aydin, A., Luft, F.C., Schachterle, C., Weise, A., Stricker, S., Lindschau, C., Vaegler, M., Qadri, F., Toka, H.R., et al. (2015). PDE3A mutations cause autosomal dominant hypertension with brachydactyly. *Nat Genet* 47, 647-653.

49. Toka, O., Tank, J., Schachterle, C., Aydin, A., Maass, P.G., Elitok, S., Bartels-Klein, E., Hollfinger, I., Lindschau, C., Mai, K., et al. (2015). Clinical effects of phosphodiesterase 3A mutations in inherited hypertension with brachydactyly. *Hypertension* 66, 800-808.
50. Vasan, R.S., Glazer, N.L., Felix, J.F., Lieb, W., Wild, P.S., Felix, S.B., Watzinger, N., Larson, M.G., Smith, N.L., Dehghan, A., et al. (2009). Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. *JAMA : the journal of the American Medical Association* 302, 168-178.
51. Fontana, V., McDonough, C.W., Gong, Y., El Rouby, N.M., Sa, A.C., Taylor, K.D., Chen, Y.D., Gums, J.G., Chapman, A.B., Turner, S.T., et al. (2014). Large-scale gene-centric analysis identifies polymorphisms for resistant hypertension. *J Am Heart Assoc* 3, e001398.
52. Simino, J., Sung, Y.J., Kume, R., Schwander, K., and Rao, D.C. (2013). Gene-alcohol interactions identify several novel blood pressure loci including a promising locus near SLC16A9. *Front Genet* 4, 277.
53. Coan, P.M., Hummel, O., Garcia Diaz, A., Barrier, M., Alfazema, N., Norsworthy, P.J., Pravenec, M., Petretto, E., Hubner, N., and Aitman, T.J. (2017). Genetic, physiological and comparative genomic studies of hypertension and insulin resistance in the spontaneously hypertensive rat. *Dis Model Mech* 10, 297-306.
54. Hong, K.W., Go, M.J., Jin, H.S., Lim, J.E., Lee, J.Y., Han, B.G., Hwang, S.Y., Lee, S.H., Park, H.K., Cho, Y.S., et al. (2010). Genetic variations in ATP2B1, CSK, ARSG and CSMD1 loci are related to blood pressure and/or hypertension in two Korean cohorts. *J Hum Hypertens* 24, 367-372.
55. Koriyama, H., Nakagami, H., Katsuya, T., Sugimoto, K., Yamashita, H., Takami, Y., Maeda, S., Kubo, M., Takahashi, A., Nakamura, Y., et al. (2010). Identification of evidence suggestive of an association with peripheral arterial disease at the OSBPL10 locus by genome-wide investigation in the Japanese population. *J Atheroscler Thromb* 17, 1054-1062.
56. Liu, C.T., Monda, K.L., Taylor, K.C., Lange, L., Demerath, E.W., Palmas, W., Wojczynski, M.K., Ellis, J.C., Vitolins, M.Z., Liu, S., et al. (2013). Genome-wide association of body fat distribution in African ancestry populations suggests new loci. *PLoS Genet* 9, e1003681.
57. Irvin, M.R., Wineinger, N.E., Rice, T.K., Pajewski, N.M., Kabagambe, E.K., Gu, C.C., Pankow, J., North, K.E., Wilk, J.B., Freedman, B.I., et al. (2011). Genome-wide detection of allele specific copy number variation associated with insulin resistance in African Americans from the HyperGEN study. *PLoS One* 6, e24052.
58. Uhl, G.R., Drgon, T., Johnson, C., Li, C.Y., Contoreggi, C., Hess, J., Naiman, D., and Liu, Q.R. (2008). Molecular genetics of addiction and related heritable phenotypes: genome-wide association approaches identify "connectivity constellation" and drug target genes with pleiotropic effects. *Ann N Y Acad Sci* 1141, 318-381.
59. Moon, S.H., Kim, Y.J., Kim, Y.K., Kim, D.J., Lee, J.Y., Go, M.J., Shin, Y.A., Hong, C.B., and Kim, B.J. (2011). Genome-wide Survey of Copy Number Variants Associated with Blood Pressure and Body Mass Index in a Korean Population. *Genomics Informatics*, 152-160.
60. Petrovic, S., Barone, S., Xu, J., Conforti, L., Ma, L., Kujala, M., Kere, J., and Soleimani, M. (2004). SLC26A7: a basolateral Cl-/HCO3- exchanger specific to intercalated cells of the outer medullary collecting duct. *Am J Physiol Renal Physiol* 286, F161-169.
61. Henke, N., Schmidt-Ullrich, R., Dechend, R., Park, J.K., Qadri, F., Wellner, M., Obst, M., Gross, V., Dietz, R., Luft, F.C., et al. (2007). Vascular endothelial cell-specific NF-kappaB suppression attenuates hypertension-induced renal damage. *Circ Res* 101, 268-276.
62. Pierce, G.L., Lesniewski, L.A., Lawson, B.R., Beske, S.D., and Seals, D.R. (2009). Nuclear factor- κ B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation* 119, 1284-1292.

63. Duan, P., Wang, Z.M., Liu, J., Wang, L.N., Yang, Z., and Tu, P. (2015). Association of gene polymorphisms in RANKL/RANK/OPG system with hypertension and blood pressure in Chinese women. *J Hum Hypertens* 29, 749-753.
64. Woods, R.L., and Johnston, C.I. (1983). Contribution of vasopressin to the maintenance of blood pressure during dehydration. *Am J Physiol* 245, F615-621.
65. Aoyagi, T., Izumi, Y., Hiroshima, M., Matsuzaki, T., Yasuoka, Y., Sanbe, A., Miyazaki, H., Fujiwara, Y., Nakayama, Y., Kohda, Y., et al. (2008). Vasopressin regulates the renin-angiotensin-aldosterone system via V1a receptors in macula densa cells. *Am J Physiol Renal Physiol* 295, F100-107.
66. Koshimizu, T.A., Nasa, Y., Tanoue, A., Oikawa, R., Kawahara, Y., Kiyono, Y., Adachi, T., Tanaka, T., Kuwaki, T., Mori, T., et al. (2006). V1a vasopressin receptors maintain normal blood pressure by regulating circulating blood volume and baroreflex sensitivity. *Proc Natl Acad Sci U S A* 103, 7807-7812.
67. Burrell, L.M., Phillips, P.A., Risvanis, J., Aldred, K.L., Hutchins, A.M., and Johnston, C.I. (1995). Attenuation of genetic hypertension after short-term vasopressin V1A receptor antagonism. *Hypertension* 26, 828-834.
68. Ronzaud, C., Loffing-Cueni, D., Hausel, P., Debonneville, A., Malsure, S.R., Fowler-Jaeger, N., Boase, N.A., Perrier, R., Maillard, M., Yang, B., et al. (2013). Renal tubular NEDD4-2 deficiency causes NCC-mediated salt-dependent hypertension. *J Clin Invest* 123, 657-665.
69. Debonneville, C., Flores, S.Y., Kamynina, E., Plant, P.J., Tauxe, C., Thomas, M.A., Munster, C., Chraïbi, A., Pratt, J.H., Horisberger, J.D., et al. (2001). Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na(+) channel cell surface expression. *EMBO J* 20, 7052-7059.
70. Zheng, X., Morrison, A.C., Feingold, E., Turner, S.T., and Ferrell, R.E. (2011). Association between NEDD4L gene and sodium lithium countertransport. *American journal of hypertension* 24, 145-148.
71. Luo, F., Wang, Y., Wang, X., Sun, K., Zhou, X., and Hui, R. (2009). A functional variant of NEDD4L is associated with hypertension, antihypertensive response, and orthostatic hypotension. *Hypertension* 54, 796-801.
72. Svensson-Farbom, P., Wahlstrand, B., Almgren, P., Dahlberg, J., Fava, C., Kjeldsen, S., Hedner, T., and Melander, O. (2011). A functional variant of the NEDD4L gene is associated with beneficial treatment response with beta-blockers and diuretics in hypertensive patients. *J Hypertens* 29, 388-395.
73. McDonough, C.W., Burbage, S.E., Duarte, J.D., Gong, Y., Langae, T.Y., Turner, S.T., Gums, J.G., Chapman, A.B., Bailey, K.R., Beitelshes, A.L., et al. (2013). Association of variants in NEDD4L with blood pressure response and adverse cardiovascular outcomes in hypertensive patients treated with thiazide diuretics. *J Hypertens* 31, 698-704.
74. Goldberg, L.I. (1972). Cardiovascular and renal actions of dopamine: potential clinical applications. *Pharmacol Rev* 24, 1-29.
75. Li, X.X., Bek, M., Asico, L.D., Yang, Z., Grandy, D.K., Goldstein, D.S., Rubinstein, M., Eisner, G.M., and Jose, P.A. (2001). Adrenergic and endothelin B receptor-dependent hypertension in dopamine receptor type-2 knockout mice. *Hypertension* 38, 303-308.
76. Armando, I., Wang, X., Villar, V.A., Jones, J.E., Asico, L.D., Escano, C., and Jose, P.A. (2007). Reactive oxygen species-dependent hypertension in dopamine D2 receptor-deficient mice. *Hypertension* 49, 672-678.
77. Zhang, Y., Jiang, X., Qin, C., Cuevas, S., Jose, P.A., and Armando, I. (2016). Dopamine D2 receptors' effects on renal inflammation are mediated by regulation of PP2A function. *Am J Physiol Renal Physiol* 310, F128-134.
78. Yang, Y., Cuevas, S., Yang, S., Villar, V.A., Escano, C., Asico, L., Yu, P., Jiang, X., Weinman, E.J., Armando, I., et al. (2014). Sestrin2 decreases renal oxidative stress, lowers blood pressure, and

- mediates dopamine D2 receptor-induced inhibition of reactive oxygen species production. *Hypertension* 64, 825-832.
79. Yi, L., Li, F., Yong, Y., Jianting, D., Liting, Z., Xuansheng, H., Fei, L., and Jiewen, L. (2014). Upregulation of sestrin-2 expression protects against endothelial toxicity of angiotensin II. *Cell Biol Toxicol* 30, 147-156.
 80. Paisan-Ruiz, C., Guevara, R., Federoff, M., Hanagasi, H., Sina, F., Elahi, E., Schneider, S.A., Schwingenschuh, P., Bajaj, N., Emre, M., et al. (2010). Early-onset L-dopa-responsive parkinsonism with pyramidal signs due to ATP13A2, PLA2G6, FBXO7 and spatascins mutations. *Mov Disord* 25, 1791-1800.
 81. Ouimet, C.C., Hemmings, H.C., Jr., and Greengard, P. (1989). ARPP-21, a cyclic AMP-regulated phosphoprotein enriched in dopamine-innervated brain regions. II. Immunocytochemical localization in rat brain. *J Neurosci* 9, 865-875.
 82. Pierce, R.C., and Kumaresan, V. (2006). The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci Biobehav Rev* 30, 215-238.
 83. King, K.S., and Pacak, K. (2014). Familial pheochromocytomas and paragangliomas. *Mol Cell Endocrinol* 386, 92-100.
 84. Hes, F.J., Weiss, M.M., Woortman, S.A., de Miranda, N.F., van Bunderen, P.A., Bonsing, B.A., Stokkel, M.P., Morreau, H., Romijn, J.A., Jansen, J.C., et al. (2010). Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. *BMC Med Genet* 11, 92.
 85. Parmar, P.G., Taal, H.R., Timpson, N.J., Thiering, E., Lehtimäki, T., Marinelli, M., Lind, P.A., Howe, L.D., Verwoert, G., Aalto, V., et al. (2016). International Genome-Wide Association Study Consortium Identifies Novel Loci Associated With Blood Pressure in Children and Adolescents. *Circ Cardiovasc Genet* 9, 266-278.
 86. Luecke, S., Backlund, M., Jux, B., Esser, C., Krutmann, J., and Rannug, A. (2010). The aryl hydrocarbon receptor (AHR), a novel regulator of human melanogenesis. *Pigment Cell Melanoma Res* 23, 828-833.
 87. Wang, L., Chu, A., Buring, J.E., Ridker, P.M., Chasman, D.I., and Sesso, H.D. (2014). Common genetic variations in the vitamin D pathway in relation to blood pressure. *Am J Hypertens* 27, 1387-1395.
 88. Sesso, H.D., Stampfer, M.J., Rosner, B., Hennekens, C.H., Gaziano, J.M., Manson, J.E., and Glynn, R.J. (2000). Systolic and diastolic blood pressure, pulse pressure, and mean arterial pressure as predictors of cardiovascular disease risk in Men. *Hypertension* 36, 801-807.
 89. Blacher, J., Staessen, J.A., Girerd, X., Gasowski, J., Thijs, L., Liu, L., Wang, J.G., Fagard, R.H., and Safar, M.E. (2000). Pulse pressure not mean pressure determines cardiovascular risk in older hypertensive patients. *Arch Intern Med* 160, 1085-1089.
 90. Gasowski, J., Fagard, R.H., Staessen, J.A., Grodzicki, T., Pocock, S., Boutitie, F., Gueyffier, F., Boissel, J.P., and Collaborators, I.P. (2002). Pulsatile blood pressure component as predictor of mortality in hypertension: a meta-analysis of clinical trial control groups. *J Hypertens* 20, 145-151.
 91. Gambier, N., Marteau, J.B., Batt, A.M., Marie, B., Thompson, A., Siest, G., Foerzler, D., and Visvikis-Siest, S. (2006). Interaction between CYP1A1 T3801C and AHR G1661A polymorphisms according to smoking status on blood pressure in the Stanislas cohort. *J Hypertens* 24, 2199-2205.
 92. Lund, A.K., Goens, M.B., Nunez, B.A., and Walker, M.K. (2006). Characterizing the role of endothelin-1 in the progression of cardiac hypertrophy in aryl hydrocarbon receptor (AhR) null mice. *Toxicol Appl Pharmacol* 212, 127-135.
 93. Niermann, T., Schmutz, S., Erne, P., and Resink, T. (2003). Aryl hydrocarbon receptor ligands repress T-cadherin expression in vascular smooth muscle cells. *Biochem Biophys Res Commun* 300, 943-949.

94. Kuzmenko, Y.S., Kern, F., Bochkov, V.N., Tkachuk, V.A., and Resink, T.J. (1998). Density- and proliferation status-dependent expression of T-cadherin, a novel lipoprotein-binding glycoprotein: a function in negative regulation of smooth muscle cell growth? *FEBS Lett* 434, 183-187.
95. Org, E., Eyheramendy, S., Juhanson, P., Gieger, C., Lichtner, P., Klopp, N., Veldre, G., Doring, A., Viigimaa, M., Sober, S., et al. (2009). Genome-wide scan identifies CDH13 as a novel susceptibility locus contributing to blood pressure determination in two European populations. *Hum Mol Genet* 18, 2288-2296.
96. Nguyen, L.P., and Bradfield, C.A. (2008). The search for endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol* 21, 102-116.
97. Martey, C.A., Bagloli, C.J., Gasiewicz, T.A., Sime, P.J., and Phipps, R.P. (2005). The aryl hydrocarbon receptor is a regulator of cigarette smoke induction of the cyclooxygenase and prostaglandin pathways in human lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol* 289, L391-399.
98. Blacher, J., and Safar, M.E. (2005). Large-artery stiffness, hypertension and cardiovascular risk in older patients. *Nat Clin Pract Cardiovasc Med* 2, 450-455.
99. Whelton, P.K., Carey, R.M., Aronow, W.S., Casey, D.E., Jr., Collins, K.J., Dennison Himmelfarb, C., DePalma, S.M., Gidding, S., Jamerson, K.A., Jones, D.W., et al. (2017). 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Hypertension.
100. Keller, M.C. (2014). Gene x environment interaction studies have not properly controlled for potential confounders: the problem and the (simple) solution. *Biological psychiatry* 75, 18-24.
101. Tyrrell, J., Wood, A.R., Ames, R.M., Yaghootkar, H., Beaumont, R.N., Jones, S.E., Tuke, M.A., Ruth, K.S., Freathy, R.M., Davey Smith, G., et al. (2017). Gene-obesogenic environment interactions in the UK Biobank study. *International journal of epidemiology* 46, 559-575.

Figure legends

Figure 1: Study design

Summary of data included in this study.

Smk: smoking status (considering either current smoking or ever smoking status separately); PC: principal component; EUR: European; AFR: African; ASN: Asian; HIS: Hispanic; BRZ: Brazilian; TRANS; trans-ancestry (i.e., combining all ancestry groups through meta-analysis)

Figure 2: Venn diagram of loci/signals associated with the 4 BP traits

The diagram shows 133 loci and/or signals that were identified through gene-smoking interactions. We had reported 81 loci associated with SBP/DBP,¹⁴ among which 37 showed association with MAP or PP. In this paper, we newly identified 41 loci (Table 2) and 11 signals near known BP loci (Table 3) that are unique to MAP and/or PP.

SBP: systolic blood pressure; DBP, diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure

Figure 3: Smoking-specific genetic effect sizes for MAP or PP

Among the 139 loci significantly associated with MAP and/or PP, 8 loci show significant interactions with smoking exposure status in African ancestry (1 DF interaction $P < 5 \times 10^{-8}$).

Smoking-specific effect estimates and 95% confidence intervals for variants associated with BP traits are shown as red and blue squares for current-smokers and non-current smokers, respectively.

MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking

Figure 4: Smoking-specific estimates of variance explained in European ancestry

The variants with $P \leq 5 \times 10^{-8}$ explained 1.9% of variance in MAP and 0.5% of variance in PP, whereas variants with $P \leq 10^{-4}$ explained 16% of variance in MAP and 11% of variance in PP. The vertical line corresponds to FDR = 0.1.

Figure 5: LocusZoom plots for 4 selected loci associated with MAP and/or PP

(A) rs140881076 (chr1:15364113) was identified in an analysis of individuals of African ancestry and is intronic to *KAZN*; neighboring genes have been implicated in cardiovascular traits. *FHADI* is a long non-coding RNA overexpressed in heart failure, *TMEM51* has been associated with contractile function in cardiomyocytes, and *CASP9* plays a central role in cardiomyocyte apoptosis.

(B) rs140994551 (chr8:4449086), intronic to *CSMD1*, shows interaction with current smoking in individuals of African Ancestry. *CSMD1* are shown to be associated with insulin resistance and BP in the spontaneously hypertensive rats. *CSMD1* is also suggestively-associated with studies of addiction and related disorders.

(C) rs11991823 (chr8:92188440) was associated with PP in trans-ancestry analyses and is intronic to *LRRC69*. The nearby gene *SLC26A7* encodes a chloride/bicarbonate-exchanger expressed specifically in the renal outer medullary collecting duct.

(D) rs146924684 (chr12:63437286) was association with MAP in individuals of African ancestry. The nearby gene *AVPR1A* is widely expressed including in vascular smooth muscle cells, kidney, myocardium, and brain.

CurSmk: current smoking status; EverSmk: ever smoking status; MAP: mean arterial pressure; PP: pulse pressure

Tables

Table 1: Basic characteristics of cohorts in Stages 1 and 2 in each ancestry.

	Current Smoker		Former Smoker		Never Smoker		Male	HTN	HT Meds	Age		MAP		PP	
	N	%	N	%	N	%	%	%	%	Mean	SD	Mean	SD	Mean	SD
Stage 1															
EUR	14,607	18.1	28,409	35.3	37,535	46.6	32.6	38.2	25.4	54.63	8	94.63	12.9	52.02	13.3
AFR	5,545	21.5	7,185	27.8	13,121	50.8	26.5	55.9	39.5	54.49	9.1	99.96	14.9	54.67	16.4
ASN	2,465	18.3	1,677	12.5	9,296	69.2	51.2	46.9	27	55.42	9.7	98.70	13.4	57.86	15.8
HIS	1,068	12.1	2,160	24.5	5,577	63.3	24.9	43.5	13.3	55.5	11	94.80	13.9	53.55	16.4
<i>Stage 1 Total</i>	<i>23,685</i>	<i>18.4</i>	<i>39,431</i>	<i>30.7</i>	<i>65,529</i>	<i>50.9</i>	<i>32.8</i>	<i>43.1</i>	<i>27.7</i>	<i>54.74</i>	<i>8.6</i>	<i>96.17</i>	<i>13.4</i>	<i>53.28</i>	<i>14.4</i>
Stage 2															
EUR	48,198	17	89,597	31.6	145,914	51.4	47.8	44.8	25	55.91	8.6	102.17	13.5	55.29	13.9
AFR	1,971	29.8	1,579	23.8	3,075	46.4	40.9	54.3	42.8	53.66	10.2	101.21	14.7	53.68	14.8
ASN	29,485	19.8	40,850	27.4	78,597	52.8	54.9	50.3	33.1	60.76	12.3	98.31	13.9	54.91	14.0
HIS	2,739	20.3	2,559	18.9	8,231	60.8	41	26.9	16.3	45.86	13.8	91.36	13.7	48.99	13.3
BRZ	998	22.6	514	11.6	2,902	65.8	48	15.5	6.3	27.78	3.2	89.75	12.3	45.23	9.8
<i>Stage 2 Total</i>	<i>83,391</i>	<i>18.2</i>	<i>135,099</i>	<i>29.6</i>	<i>238,719</i>	<i>52.2</i>	<i>49.7</i>	<i>45.9</i>	<i>27.4</i>	<i>56.84</i>	<i>9.9</i>	<i>100.54</i>	<i>13.7</i>	<i>54.88</i>	<i>13.9</i>
TOTAL	107,076	18.3	174,530	29.8	304,248	51.9	46.1	45.3	27.4	56.4	9.6	99.61	13.6	54.54	14.0

The cell entries for the covariates and BP traits correspond to sample-size weighted averages across all cohorts in each category. EUR: European; AFR: African; ASN: Asian; HIS: Hispanic; BRZ: Brazilian; ALL: trans-ancestry (i.e., combining all ancestry groups through meta-analysis); HTN: hypertension; MAP: mean arterial pressure; PP: pulse pressure

Table 2: Forty-one new loci associated with MAP and/or PP that are at least 1Mb away from any known BP locus

Locus	rsID	Nearest Gene	Pos	Freq	Race	Trait/ Exposure	Effect	StdErr	GxE Effect	GxE StdErr	Interaction P	Joint P	FDR q value
1	rs115356163	<i>PADI2</i>	1:17466024	0.02	AFR	PP/CS	0.22	0.87	-7.70	1.53	0.04	5.17E-09	3.63E-05
2	rs147515295	<i>EYA3; SESN2</i>	1:28389841	0.98	HIS	MAP/ES	2.94	1.04	2.80	1.52	0.10	3.47E-08	0.018721
3	rs11587661	<i>COG2</i>	1:230671208	0.02	AFR	PP/CS	0.44	0.86	-7.63	1.51	1.31E-06	4.95E-08	0.010168
4	rs138318054	<i>KIAA1804</i>	1:233578559	0.02	AFR	PP/CS	-0.37	0.93	-7.58	1.66	1.40E-05	4.84E-08	0.010095
5	rs79113694	<i>GALNT14</i>	2:31253799	0.03	AFR	PP/ES	-0.60	0.58	-2.91	0.83	1.98E-04	7.65E-09	5.96E-05
6	rs183927068	<i>MAP2</i>	2:210288479	0.98	AFR	MAP/CS	-0.60	1.09	11.29	2.02	8.36E-08	2.05E-09	0.001619
7	rs75875736	<i>STAC</i>	3:36341106	0.02	AFR	PP/ES	-3.49	0.58	3.15	0.94	1.23E-03	1.41E-08	0.000108
8	rs116199364	<i>CLSTN2</i>	3:139951198	0.02	AFR	PP/CS	1.94	0.92	-10.54	1.88	2.23E-08	1.04E-07	0.000675
9	rs114619985	<i>BOD1L</i>	4:13599930	0.02	AFR	PP/ES	-2.74	0.78	-1.86	1.13	0.04	2.71E-10	2.61E-06
10	rs201223145	<i>PRDM5</i>	4:121706475	0.97	AFR	PP/CS	2.67	0.68	2.91	1.39	0.12	5.91E-09	0.001905
11	rs147998309	<i>PCDH10</i>	4:133596832	0.99	AFR	PP/CS	1.61	1.18	12.94	2.64	1.78E-06	2.41E-09	1.74E-05
12	rs146622638	<i>GPM6A</i>	4:176524533	0.97	AFR	PP/ES	2.76	0.65	0.16	0.98	0.95	4.55E-08	0.000334
13	rs72723039	<i>IRX2</i>	5:2664169	0.98	AFR	PP/CS	-1.69	1.10	10.76	1.88	2.39E-08	6.55E-09	0.002064
14	rs79205226	<i>CDKALI</i>	6:21103825	0.02	AFR	PP/CS	1.46	0.68	-7.94	1.30	1.60E-09	3.38E-09	2.41E-05
15	rs200495667	<i>ALDH8A1</i>	6:135152480	0.08	ASN	PP/CS	-2.48	0.41	2.63	0.92	3.11E-03	1.50E-08	0.000378
16	rs190090939	<i>ACTR3B</i>	7:152802243	0.01	AFR	PP/CS	-0.01	1.12	-11.94	2.24	1.86E-07	5.41E-09	3.79E-05
17	rs140994551	<i>CSMD1</i>	8:4449086	0.01	AFR	PP/CS	0.43	1.07	-11.39	1.89	4.34E-09	2.07E-11	1.93E-07
18	rs4841126	<i>LOC157273</i>	8:9144610	0.45	EUR	MAP/ES	-0.24	0.04	0.10	0.06	0.11	3.59E-09	1.11E-05
19	rs6601302	<i>LOC157273</i>	8:9239458	0.25	EUR	MAP/ES	0.25	0.05	-0.08	0.07	0.32	6.94E-09	2.11E-05
20	rs7817784	<i>TNKS</i>	8:9682553	0.57	EUR	MAP/CS	-0.23	0.03	0.05	0.08	0.89	6.93E-13	2.59E-09
21	rs17151108	<i>MSRA</i>	8:9971119	0.26	EUR	MAP/CS	0.20	0.04	0.03	0.09	0.63	1.13E-08	3.26E-05
22	rs12156238	<i>FAM167A</i>	8:11285135	0.19	EUR	MAP/ES	-0.30	0.06	0.10	0.08	0.29	1.03E-08	1.69E-05
23	MERGED_DEL _2_50178		8:79178179	0.01	EUR	MAP/CS	1.60	1.34	-9.18	1.86	6.30E-07	1.25E-08	3.56E-05
24	rs11991823	<i>LRRC69; SLC26A7</i>	8:92188440	0.37	Trans	PP/ES	-0.23	0.03	0.06	0.05	0.43	1.29E-15	8.89E-11
25	rs7823377	<i>TRHR</i>	8:110073120	0.59	EUR	PP/CS	-0.15	0.03	0.02	0.08	0.86	5.80E-06	0.00026
26	rs76209156	<i>KDM4C</i>	9:7423109	0.99	AFR	PP/CS	0.03	1.19	10.43	2.14	1.96E-06	2.94E-08	0.000197

27	rs77548020	<i>FLJ41200;</i> <i>NFIB</i>	9:13480744	0.98	AFR	PP/CS	0.75	0.83	7.27	1.59	1.56E-04	1.91E-08	0.00013
28	rs75872665	<i>LOC100128811</i>	10:25388468	0.99	AFR	PP/CS	0.08	1.09	8.94	1.88	3.41E-04	2.80E-08	0.000188
29	rs76497600	<i>BUB3</i>	10:125119610	0.03	AFR	PP/ES	-0.79	0.61	-2.65	0.85	0.01	2.29E-08	0.000173
30	rs148454833	<i>OR52A4</i>	11:5114798	0.98	AFR	PP/CS	0.34	0.76	7.09	1.47	8.39E-06	2.16E-08	0.000147
31	rs186331780	<i>FAM19A2</i>	12:61710810	0.02	AFR	PP/CS	-2.43	0.89	-4.88	1.66	0.02	3.15E-08	0.007099
32	rs146924684	<i>AVPR1A</i>	12:63437286	0.99	AFR	MAP/ES	4.88	0.83	-3.20	1.22	0.18	5.29E-09	2.62E-05
33	rs117206641	<i>FBRSL1</i>	12:133086888	0.11	Trans	MAP/CS	0.32	0.05	0.03	0.13	0.70	1.14E-10	5.71E-07
34	rs73212161	<i>TDRD3</i>	13:61261485	0.99	AFR	PP/ES	-1.39	1.40	7.80	1.77	1.50E-05	1.68E-08	0.006503
35	rs78265647	<i>IGF1R</i>	15:99247941	0.98	AFR	PP/CS	-1.71	0.72	7.64	1.28	2.02E-09	8.86E-09	6.09E-05
36	rs145181522	<i>TOX3</i>	16:52490106	0.02	AFR	PP/CS	-0.65	0.95	-8.04	1.58	3.67E-05	3.66E-11	3.32E-07
37	rs114511313	<i>NUDT7</i>	16:77706251	0.98	AFR	PP/CS	1.67	0.73	4.06	1.28	0.13	1.63E-08	0.000111
38	rs75129914	<i>RIT2</i>	18:40267945	0.97	AFR	PP/ES	0.32	0.61	3.42	0.85	3.81E-04	2.13E-09	1.80E-05
39	rs115134409	<i>MALT1;</i> <i>NEDD4L</i>	18:56324467	0.02	AFR	PP/CS	-0.31	0.77	-6.46	1.29	3.26E-03	3.64E-10	2.92E-06
40	rs78375085	<i>TNFRSF11A</i>	18:60032891	0.98	AFR	PP/ES	4.55	0.77	-5.57	1.21	4.71E-06	1.64E-08	0.000124
41	rs191056303	<i>PXMP4</i>	20:32306802	0.98	AFR	PP/CS	0.15	0.74	7.41	1.47	5.99E-07	1.77E-08	0.000121

Each locus was determined through LD-based clumping, using ± 1 Mb around index variants, followed by LD threshold of $r^2 > 0.1$. Each locus is genome-wide significant ($P < 5 \times 10^{-8}$) in the combined analyses of Stages 1 and 2 and had FDR q value < 0.05 . Positions are based on human genome build 37. EA: effect allele; EAF: effect allele frequency; 2 DF Joint P: P-value of the joint test with 2 degrees of freedom of genetic main and interaction effects. 1 DF Interaction P: P-value of the interaction test with 1 degree of freedom; EUR: European ancestry. ALL: trans-ancestry (i.e., combining all ancestry groups through meta-analysis); MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking; ES: ever-smoking.

Table 3: Eleven new signals associated with MAP and/or PP that are near known BP loci (but not in LD, $r^2 < 0.1$).

Locus	rsID	Nearest Gene	Pos	Freq	1 Race	Trait/ Exposure	Effect	StdErr	GxE Effect	GxE StdErr	Interaction P	Joint P	FDR q value
1	rs140881076	<i>KAZN</i>	1:15364113	0.01	AFR	PP/CS	0.45	1.13	-11.95	1.85	2.30E-03	3.29E-14	4.16E-10
2	rs2071405	<i>AGT</i>	1:230850658	0.13	Trans	MAP/CS	0.28	0.04	-0.18	0.09	0.20	3.02E-12	1.62E-08
3	rs143802076	<i>C3orf38</i>	3:88646080	0.01	AFR	PP/CS	-0.50	0.90	-8.54	1.68	8.97E-04	1.33E-09	9.81E-06
4	rs1009382	<i>TNXB</i>	6:32026107	0.71	EUR	PP/CS	0.26	0.04	-0.16	0.08	0.15	4.84E-13	3.30E-09
5	rs7005363	<i>MSRA</i>	8:10283748	0.54	EUR	MAP/ES	-0.34	0.04	0.15	0.06	0.02	3.13E-17	1.59E-13
6	rs187148391	<i>TXN</i>	9:112998518	0.99	HIS	MAP/ES	0.09	0.69	4.48	1.03	1.01E-03	1.95E-08	0.013302
7	rs10894198	<i>ADAMTS8</i>	11:130285493	0.38	Trans	PP/CS	0.27	0.03	-0.12	0.07	0.33	1.38E-19	3.19E-15
8	rs113328593	<i>SNX19</i>	11:130742179	0.02	AFR	PP/CS	-3.06	0.79	-2.74	1.41	0.10	2.23E-09	1.60E-05
9	rs1010064	<i>LOC100506393</i> <i>PDE3A</i>	12:20000315	0.75	Trans	MAP/ES	0.24	0.04	-0.12	0.06	0.03	5.91E-11	6.64E-10
10	rs201977809	<i>LOC338758</i>	12:90095795	0.71	EUR	MAP/ES	-0.26	0.08	-0.18	0.12	0.22	1.32E-09	4.20E-06
11	rs201028933	<i>LOC338758</i>	12:90111249	0.79	Trans	MAP/ES	0.32	0.08	0.16	0.11	0.28	1.73E-11	9.75E-08

Each locus was determined through LD-based clumping, using ± 1 Mb around index variants, followed by LD threshold of $r^2 > 0.1$. Each locus is genome-wide significant ($P < 5 \times 10^{-8}$) in the combined analyses of Stages 1 and 2 and had FDR q value < 0.05 . Positions are based on human genome build 37. EA: effect allele; EAF: effect allele frequency; 2 DF Joint P: P-value of the joint test with 2 degrees of freedom of genetic main and interaction effects. 1 DF Interaction P: P-value of the interaction test with 1 degree of freedom; EUR: European ancestry. ALL: trans-ancestry (i.e., combining all ancestry groups through meta-analysis); MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking; ES: ever-smoking.

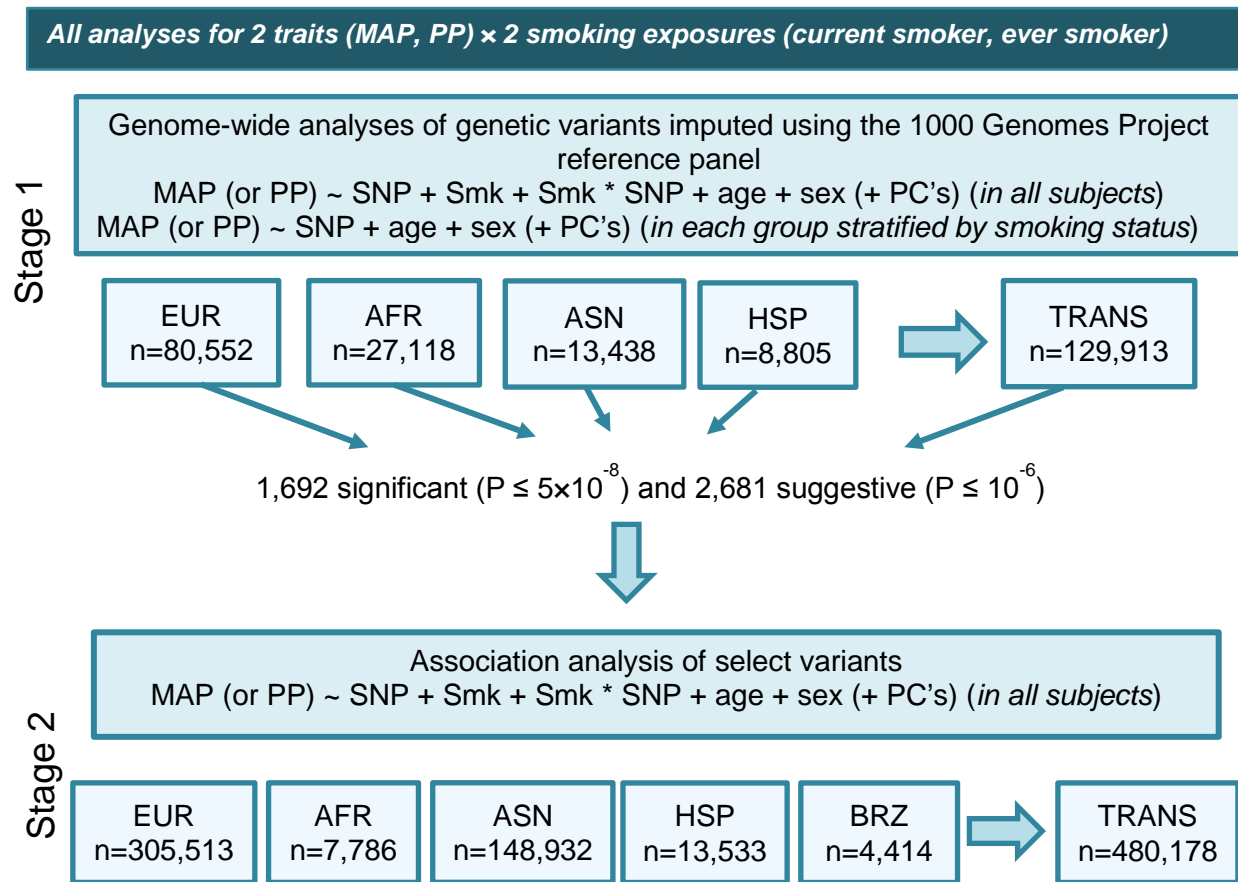


Figure 1: Study design

Summary of data included in this study.

Smk: smoking status (considering either current smoking or ever smoking status separately); PC: principal component; EUR: European; AFR: African; ASN: Asian; HIS: Hispanic; BRZ: Brazilian; TRANS; trans-ancestry (i.e., combining all ancestry groups through meta-analysis)

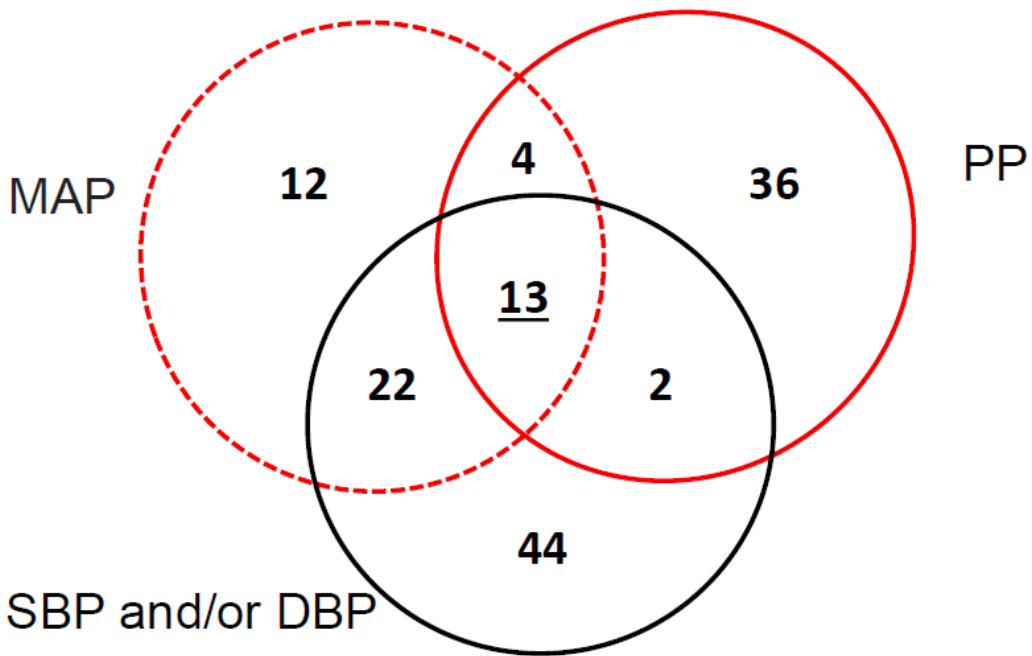


Figure 2: Venn diagram of loci/signals associated with the 4 BP traits

The diagram shows 133 loci and/or signals that were identified through gene-smoking interactions. We had reported 81 loci associated with SBP/DBP,¹⁴ among which 37 showed association with MAP or PP. In this paper, we newly identified 41 loci (Table 2) and 11 signals near known BP loci (Table 3) that are unique to MAP and/or PP (to a total of 52 new loci/signals).

SBP: systolic blood pressure; DBP, diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure

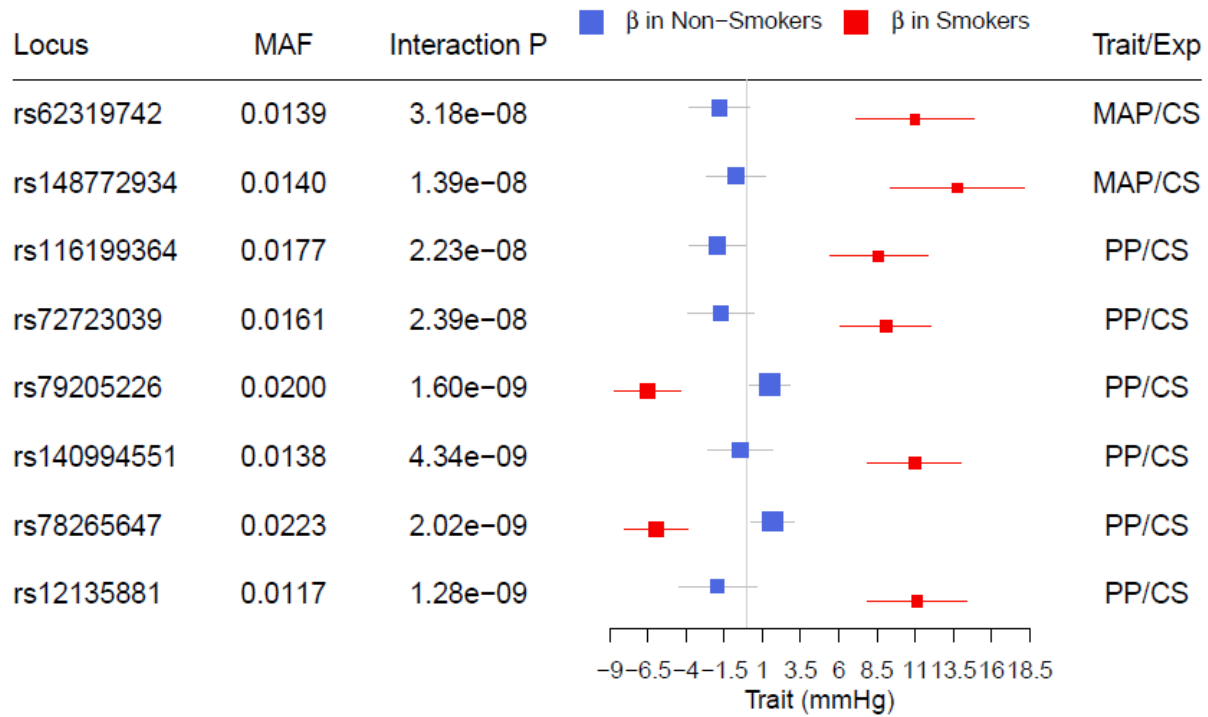


Figure 3: Smoking-specific genetic effect sizes for MAP or PP

Among the 139 loci significantly associated with MAP and/or PP, 8 loci show significant interactions with smoking exposure status in African ancestry (1 DF interaction $P < 5 \times 10^{-8}$). Smoking-specific effect estimates and 95% confidence intervals for variants associated with BP traits are shown as red and blue squares for current-smokers and non-current smokers, respectively.

MAP: mean arterial pressure; PP: pulse pressure; CS: current-smoking

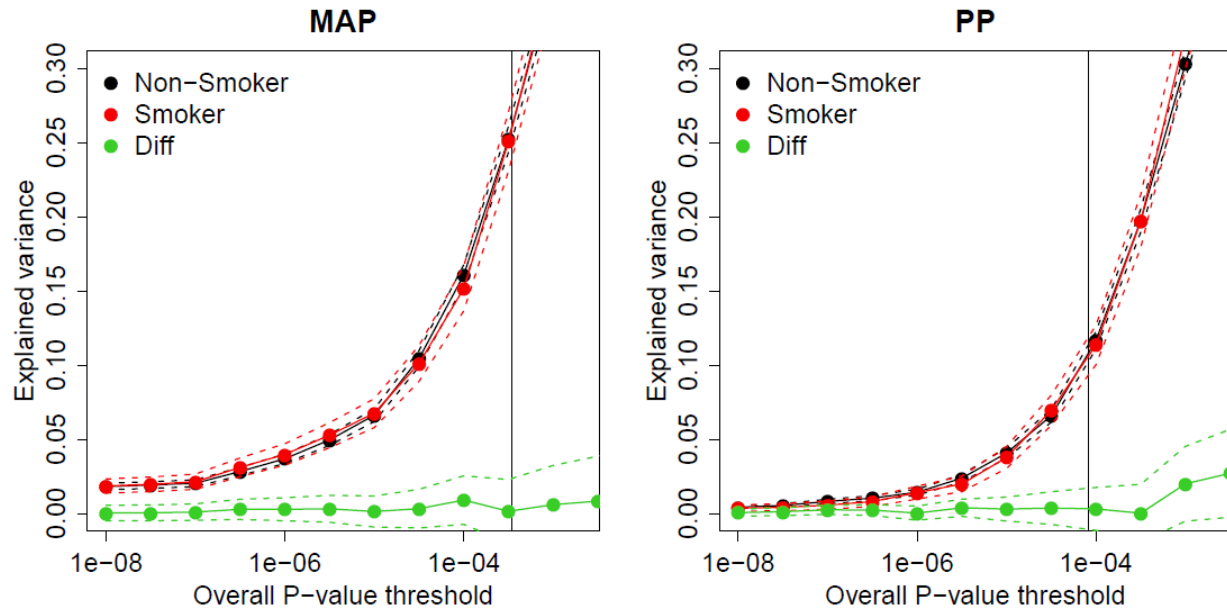


Figure 4: Smoking-specific estimates of variance explained in European ancestry

The variants with $P \leq 5 \times 10^{-8}$ explained 1.9% of variance in MAP and 0.5% of variance in PP, whereas variants with $P \leq 10^{-4}$ explained 16% of variance in MAP and 11% of variance in PP. The vertical line corresponds to FDR = 0.1.

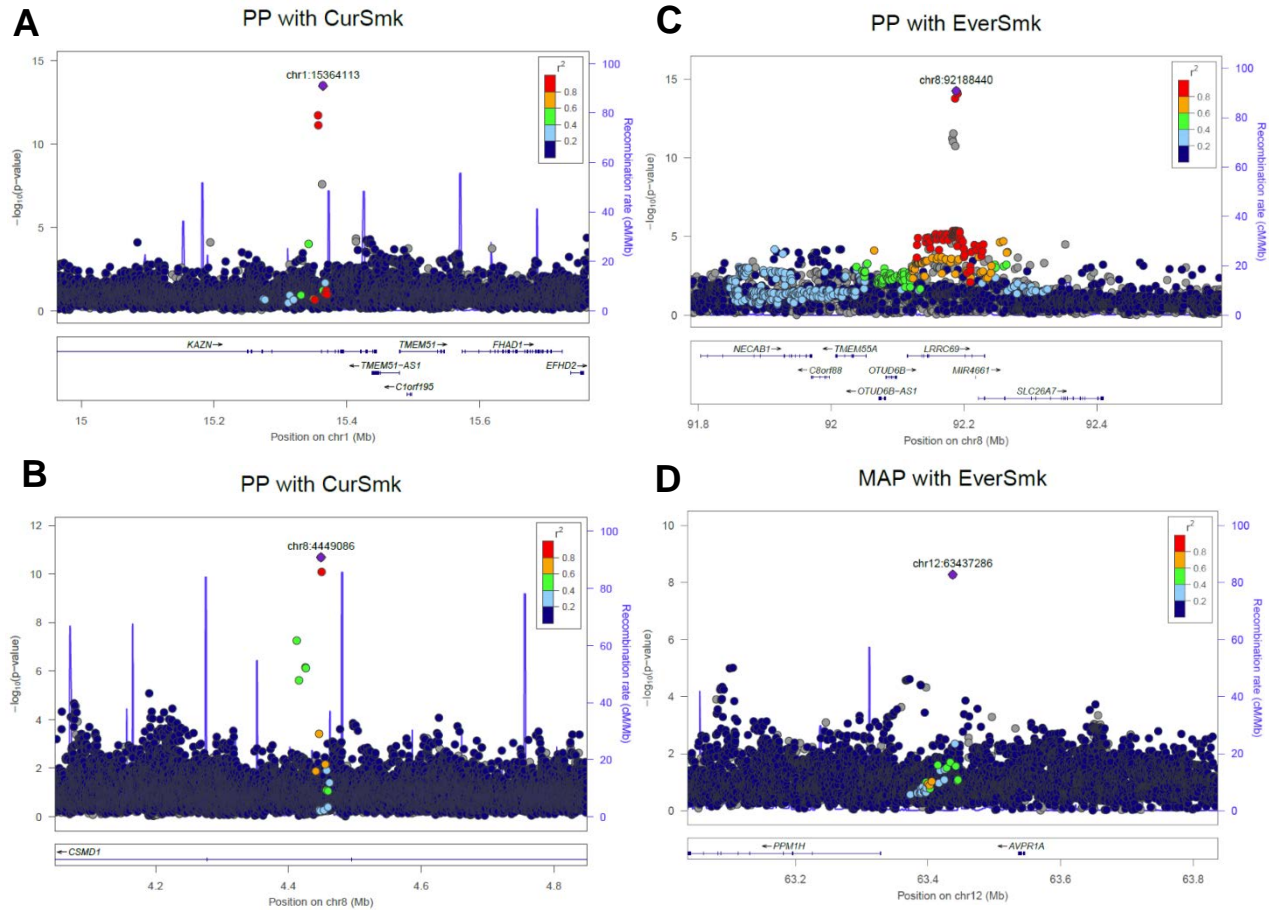


Figure 5: LocusZoom plots for 4 selected loci associated with MAP and/or PP

(A) rs140881076 (chr1:15364113) was identified in an analysis of individuals of African ancestry and is intronic to *KAZN*; neighboring genes have been implicated in cardiovascular traits. *PHAD1* is a long non-coding RNA overexpressed in heart failure, *TMEM51* has been associated with contractile function in cardiomyocytes, and *CASP9* plays a central role in cardiomyocyte apoptosis.

(B) rs140994551 (chr8:4449086), intronic to *CSMD1*, shows interaction with current smoking in individuals of African Ancestry. *CSMD1* are shown to be associated with insulin resistance and BP in the spontaneously hypertensive rats. *CSMD1* is also suggestively-associated with studies of addiction and related disorders.

(C) rs11991823 (chr8:92188440) was associated with PP in trans-ancestry analyses and is intronic to *LRRC69*. The nearby gene *SLC26A7* encodes a chloride/bicarbonate-exchanger expressed specifically in the renal outer medullary collecting duct.

(D) rs146924684 (chr12:63437286) was association with MAP in individuals of African ancestry. The nearby gene *AVPR1A* is widely expressed including in vascular smooth muscle cells, kidney, myocardium, and brain.

CurSmk: current smoking status; EverSmk: ever smoking status; MAP: mean arterial pressure; PP: pulse pressure

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